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Between Thoughts and Actions: Motivationally Salient Cues Invigorate Mental Action in the Human Brain

Avi Mendelsohn,[1] Alex Pine,[2] and Daniela Schiller[1,*]
[1]Departments of Psychiatry and Neuroscience, Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA
[2]Department of Neurobiology, Weizmann Institute of Science, 7610001 Rehovot, Israel
*Correspondence: daniela.schiller@mssm.edu
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SUMMARY

The maintenance of goal-directed behavior relies upon a cascade of covert mental actions including motor imagery and planning. Here we investigated how cues imbued with motivational salience can invigorate motor imagery networks preceding action. We adapted the Pavlovian-to-instrumental (PIT) paradigm to explore this by substituting motor action with motor imagery. Thus, reward was contingent upon a given level of imagery-induced neural activity using real-time fMRI. We found that the concomitant presentation of reward-related cues during motor imagery not only enhanced neural responses in motivational centers (ventral striatum and extended amygdala) but also exerted a motivational effect in the imagery network itself. Moreover, functional connectivity between ventral striatum (but not extended amygdala) and motor cortex was heightened during imagery in the presence of the reward-related cue. The concurrent activation of "value" and "action" networks may illuminate the neural process that links motivational cues to desires and urges to obtain goals.

INTRODUCTION

Desires, urges, and wishes pertinent to obtaining rewards are key components in maintaining goal-directed behavior (Wise and Rompré, 1989; Brown and Pluck, 2000; Bray et al., 2010). These mental processes can turn disruptive, such that individuals become subject to intrusive and unwanted thoughts about obtaining desired rewards (Kavanagh et al., 2009; May et al., 2010). Intrusive thoughts regarding the procurement of rewards are predictive of substance abuse relapse and binge eating and are strongly encouraged by the presence of environmental cues associated with these rewards (Everitt et al., 2001; May et al., 2010). Although the potentiating effect that environmental cues exert on actual behavior is well documented (Balleine and Killcross, 2006; Crombag et al., 2008), the neural mechanism by which motivational cues influence preparatory activity leading to action execution is unclear. We set out to examine the hypothesis that motivationally salient cues can directly influence imagery of an action by regulating the level of neural activity in motor networks that support action execution.

Environmental cues, which are initially motivationally neutral, can acquire incentive value through Pavlovian conditioning, whereby an association is formed between a neutral stimulus (NS) and a biologically significant stimulus (Pavlov, 1927). A powerful model for studying the influence that Pavlovian cues exert on goal-directed behavior is Pavlovian-to-instrumental transfer (PIT; Estes, 1948; Rescorla and Solomon, 1967; Lovibond, 1983). The PIT phenomenon is the outcome of two distinct associative learning processes, whereupon individuals form a Pavlovian association between a neutral cue and a rewarding outcome, and another association between an instrumental action and a similar reward. The rewarded cues can then activate motivational systems, which engender the potentiation of the instrumental (goal-directed) action (Dickinson and Balleine, 1994). This model can thus explain how environmental cues, associated, for example, with a drug's incentive value, may invigorate drug-seeking behavior, leading to relapse (Everitt et al., 2001; Cardinal et al., 2003).

Animal studies have shown that a distributed set of brain regions is necessary for the expression of PIT, including the amygdala, ventral striatum (Cardinal et al., 2003; Corbit and Balleine, 2005), and ventral tegmental area (Murschall and Hauber, 2006), supported by dopaminergic pathways (Lex and Hauber, 2008). Studies in humans have corroborated animal findings, pointing to the involvement of the amygdala and ventral striatum in PIT effects (Bray et al., 2008; Talmi et al., 2008; Prévost et al., 2012). These regions may mediate the PIT effect through their role in assigning motivational significance to Pavlovian stimuli and in turn affect action selection and execution (Everitt et al., 2001; Holmes et al., 2010). In the present study, we sought to examine how motivational cues may affect the neural substrates of a covert mental process, namely imagery, which precedes motor action. Toward this aim, we modified the PIT paradigm by substituting the physical action in the instrumental task with a motor imagery task.

Reinforcing a mental process such as motor imagery poses a challenge as to the means by which to measure and quantify the process upon which the reinforcement is contingent. To meet this challenge, we used a real-time fMRI technique, permitting us to monitor, online, the activation level in a particular brain

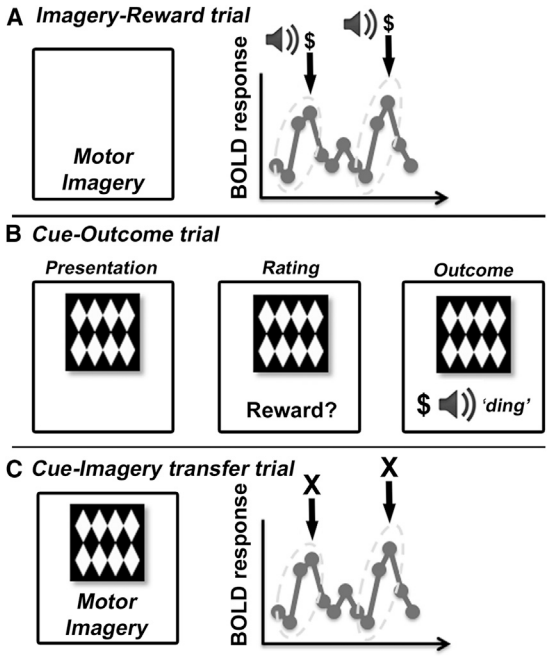

Figure 1. Experimental Design

(A) After defining a region of interest (ROI) for each participant, the Imagery-Reward stage was carried out, in which participants were required to engage in motor imagery (20–24 s) when hearing the word "up" or count backward from 200 by three (8–14 s) when hearing the word "down" (in alternating trials; ten trials each). During imagery trials, each time the BOLD signal in the defined ROI increased during two consecutive TRs, participants were rewarded with three shekels and were accordingly informed by a "ding" sound.

(B) In the following Cue-Outcome stage, two cues were presented (total trial time 6 s; 33 trials each), one predicting a monetary gain of five shekels in 40% of the trials (Gain; depicted in the figure), and one predicting loss of three shekels in 40% of the trials (Loss). Participants were asked to rate the degree to which they expected a reward. The reward was presented on the screen accompanied by a "ding" sound.

(C) Finally, participants underwent the Cue-Imagery transfer test, in which they were required to carry out the same imagery and counting tasks as in the Imagery-Reward stage (under extinction conditions) and were concurrently presented in each imagery trial with either the Gain, Loss (from the Cue-Outcome stage), or a neutral stimulus (NS), not previously presented (15 trials each, 8 s; intermixed with 45 counting trials, 4–6 s). The figure depicts a Gain trial.

area throughout the course of a scan. We first identified a subject-specific brain region activated by a motor-imagery task and subsequently formed an association between its activation level during imagery and reward receipt using real-time fMRI. Next, we associated visual cues with either a similar reward or an aversive outcome using a Pavlovian conditioning protocol. Finally, we examined the influence of the Pavlovian cues on the activation of motor-imagery and motivational networks. Our working hypothesis was that the motivational cue (referring throughout to the reward-related cue) would activate reward-related regions and that engaging in motor imagery would recruit motor networks. We further hypothesized that the concomitant presentation of the reward-related cue during motor imagery would act to enhance activation in both networks: those coding the incentive value and those involved in motor imagery, forming a synchronized pattern of activation among these systems. Such a mechanism may explain the means by which motivationally salient cues potentiate activation in centers involved in motor imagery and planning, which in turn may influence action execution.

RESULTS

Our study comprised four stages: the first stage, *Functional Localizer*, was designed to determine a region of interest (ROI) by asking participants to either imagine moving their right hand (*imagery*) or count backward from 200 in steps of three (*counting*). During the second stage, *Imagery-Reward*, participants again performed the imagery and counting tasks but now they were rewarded for successfully increasing the imagery-related brain activity in the ROI defined by the previous stage. Next, the *Cue-Outcome* stage was performed, in which participants formed one association between an initially neutral cue (e.g., striped pattern) and monetary reward (hereafter *Gain*) and another association between a cue (e.g., checkered pattern) and monetary loss (*Loss*). Finally, in the *Cue-Imagery transfer test*, participants carried out the imagery and counting tasks again. This time, in order to examine the effects of reward-related cues on neural imagery responses (associated with the same reward), participants were concomitantly presented on each imagery trial with one of the conditioned stimuli (CSs; Gain, Loss, or a new neutral cue), without receiving reward (see Figure 1).

Functional Localizer: Imagery-Related Motor Regions

In the Functional Localizer, an ROI was detected for each participant in the primary or secondary motor cortex, within which BOLD activity was higher during imagery than counting (see Figure S1 and Table S1 available online). The reward that participants received in the following Imagery-Reward stage was provided on the basis of BOLD activity in each participant's ROI (see Experimental Procedures). To examine imagery-related activation at the group level, we performed a second level analysis on the functional data sets of the Functional Localizer stage by performing an imagery > counting contrast, yielding activation in the motor cortex (left precentral gyrus; peak voxel x, y, z coordinates: −13, −14, 51), left inferior parietal lobule (−46, −59, 12), and ventrolateral prefrontal cortex (VLPFC; −40, 28, −9; see Figure 2B, left, and Table S2).

Cue-Outcome Stage: Explicit and Implicit Measures of Pavlovian Learning

In order to form appetitive and aversive associations between neutral cues and monetary gains and losses, we used a partial reinforcement Pavlovian learning protocol (Cue-Outcome stage). Learning was assessed both by explicit (estimates of cue-outcome contingencies) and implicit (skin conductance response [SCR]) measurements. Explicit ratings showed a clear divergence over trials between outcome expectancies to Gain and Loss cues, yielding higher reward-expectancy ratings for Gain trials (analysis of covariance [ANCOVA] CS × trial interaction effect: $F(1,36) = 6.51$, $p = 0.013$; Figure 3A). Pavlovian learning was also detected in SCR measures, demonstrated by

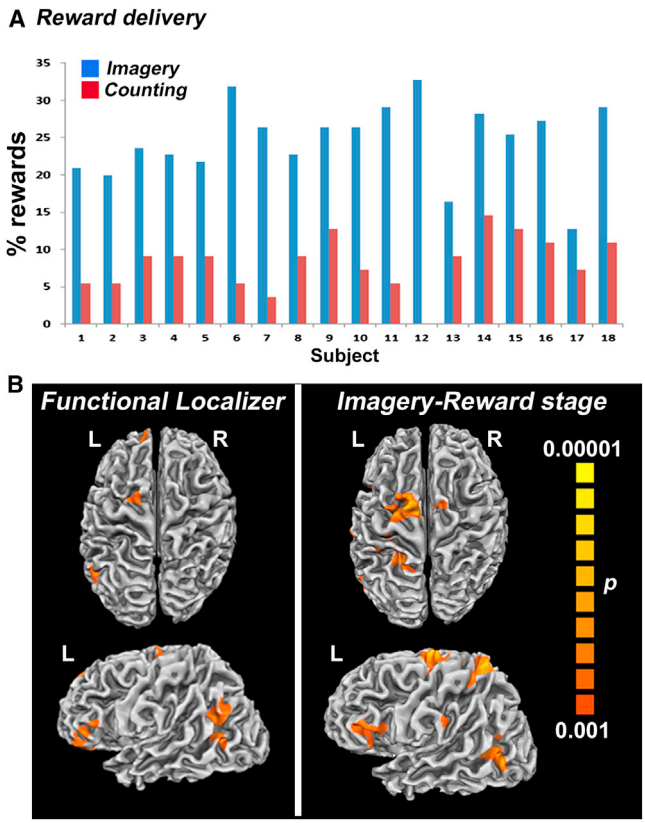

Figure 2. Motor Imagery Induced Brain Activation

(A) Bars represent the percentage of the number of TRs in which participants received a reward during the imagery trials (blue) of the Imagery-Reward stage and the percentage of TRs in which participants would have received reward during the counting task (red), had reward been delivered in these trials. The criterion for receiving a reward was based on the BOLD activation of the ROI detected in the Functional Localizer stage. All participants met the reward criterion more times during imagery than during counting (group t test: $t(17) = 10.6$, $p < 10^{-7}$).

(B) Statistical maps of brain activation during the Functional Localizer stage (left) and Imagery-Reward stage (right), depicting the results of the imagery versus counting contrasts. In the Functional Localizer stage, activations are observed in left precentral gyrus, as well as left inferior parietal lobule and left ventrolateral prefrontal cortex ($p < 0.001$, corrected for cluster size threshold at $p < 0.05$). These regions were activated to a larger extent in the Imagery-Reward stage during prereward motor-imagery trials, in addition to the right precentral gyrus, left superior parietal lobule, and striatum (see also Table S3).

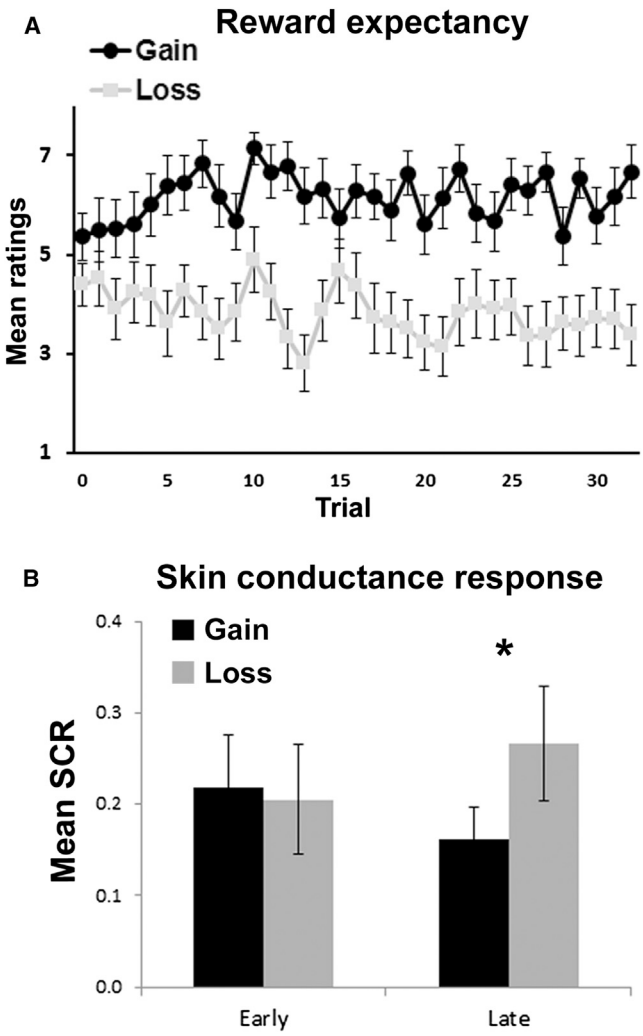

Figure 3. Cue-Outcome Stage

(A) Mean ratings of reward expectancy for each Gain (black) and Loss (gray) trial throughout the scan. Ratings for the two CSs diverged over trials, showing that participants acquired separate cue-reward and cue-loss associations. Error bars here and below represent SEM.

(B) Average skin conductance response (SCR) for early and late phases of Gain (black) and Loss (gray) trials. Participants expressed higher SCRs during Loss trials in the late conditioning phase, demonstrating a physiological aversion response to the loss-associated cue acquired over time. The asterisk represents a significant interaction effect in an ANOVA test using cue type (Gain/Loss) and trial phase (early/late) as repeated-measure factors.

a rise in SCR during presentation of Loss cues in the late learning (second half) phase compared to Gain ($t(17) = 2.22$, $p < 0.05$; Figure 3B). A repeated-measures ANOVA analysis using cue type (Gain/Loss) and time (early/late) as factors yielded a main effect for cue type across time, indicating higher SCRs to Loss compared to Gain cues ($F(1,17) = 5.1$, $p < 0.05$). Additionally, activation in ventromedial prefrontal cortex (peak x, y, z = 11, 34, −6) was detected in a contrast comparing late versus early Gain trials (Figure S2). Thus, both implicit and explicit measurements indicated that the Pavlovian associations were acquired, providing the prerequisites for meaningful assessment of potential cue-imagery transfer effects as discussed below.

Imagery-Reward Stage: Reward Delivery Contingent upon Imagery-Induced BOLD Response

During the Imagery-Reward stage, we provided each participant with a monetary reward of three Israeli shekels (~$1) each time the mean BOLD activity in the participant's motor-imagery ROI (as detected in the Functional Localizer) increased over two consecutive repetition times (TRs), thus forming an association between motor imagery and monetary gain (Bray et al., 2007). The average percentage of TRs in which participants received reward during imagery trials (number of reward divided by total number of TRs in each condition) was 24.6 ± 1.18 (Figure 2A).

In contrast, during counting trials, in which subjects were requested to count backward, offline reward analysis (i.e., calculating the percentage of TRs in which participants would have received a reward for two consecutive increases in BOLD response had a reward been provided in these trials), yielded an average of 8.18 ± 0.82. Thus, participants were successful in enhancing the motor imagery ROI during imagery trials, exhibiting considerably more consecutive increases than during counting ($t(17) = 10.6$, $p < 10^{-7}$).

To exclude BOLD responses to the reward delivery itself (i.e., activation associated with monetary reward), the statistical map was computed based on a general linear model (GLM) that included a regressor that modeled BOLD response during prereward TRs, which was compared to activation during counting. Significant activity for imagery > counting was found in a set of regions, which throughout the manuscript we will refer to as the *motor-imagery network*. This network included: bilateral motor cortex (left precentral gyrus: −7, −14, 57; right precentral gyrus: 11, −14, 60), two loci in left superior parietal lobule (−22, −50, 60; −34, −41, 51), left middle temporal gyrus (−52, −68, 0), bilateral inferior frontal gyrus (left IFG: −34, 28, −3; right IFG: 26, 31, 0), bilateral fusiform gyrus (left: −25, −41, −12; right: 20, −44, −15), as well as in subcortical regions of right dorsal caudate (17, 1, 21), left putamen (−31, −5, 18), right ventral caudate (8, 1, 3), and right cerebellar tonsil (Figure 2B, right; Table S3). The results of a counting > imagery contrast is depicted in Figure S3.

Cue-Imagery Transfer Test: The Effect of Gain and Loss Cues on Imagery-Induced BOLD Response

We have thus far provided evidence for the formation of Pavlovian learning of appetitive and aversive associations between visual cues and monetary Gain and Loss, respectively, and an "instrumental" association in which motor imagery was performed in order to obtain monetary reward. Our main objective was to examine whether and how the presence of the Pavlovian cues affected neural responses induced by motor imagery. We examined this in three steps: first, we tested the effect of Gain and Loss cues specifically on the motor-imagery network, which was identified in the Reward-Imagery stage. Second, we conducted a whole-brain analysis to identify regions outside the motor-imagery network, which were differentially affected by the Gain and Loss cues while performing motor imagery. Third, we inspected whether the presence of Gain and Loss cues during imagery affected the functional connectivity between reward and motor regions.

The Effect of Gain and Loss Cues on the Motor-Imagery Network

In the Cue-Imagery transfer test, the motor-imagery network as a whole demonstrated enhanced activity when imagery was carried out concomitant with the presentation of the Gain compared to the Loss cue (Figure 4A). This is apparent by the accumulation of data points above the dashed line, each dot representing the average beta values for Gain and Loss trials of a particular ROI within the network. Accordingly, average network activity was higher for Gain versus Loss ($t(13) = 6.57$, $p < 0.00005$; Figure 4C). A repeated-measures ANOVA analysis, using stimulus type (Gain/Loss) and region as repeated-measures factors revealed a main effect for stimulus type ($F(1,17) = 4.78$, $p < 0.05$) but not an interaction effect, implying that areas of the imagery network contributed to the Gain > Loss effect in a homogeneous manner. Importantly, the increase in network activity was not due to an overall effect of arousal, since it was specific to the presentation of Gain but not Loss cues. This validation is especially compelling given that Loss cues elicited stronger SCR than Gain cues in the Pavlovian stage. Moreover, this finding was not generalized to other networks; when examining the activation during imagery in a control "counting network," which was comprised of regions that were more activated in the counting versus imagery tasks, no such differences were found between Gain versus Loss ($t(7) = 0.92$, not significant [N.S.]; Figures 6B and 6C). To conclude, the network of brain regions that was engaged in motor imagery, as detected in the Imagery-Reward stage, exhibited an enhancement in activation upon the presentation of the reward-related CS compared to the loss-related CS, demonstrating an invigorating effect of motivational CSs on the motor-imagery network.

It should be noted that the activation level in the left motor cortex (left precentral gyrus), which was the ROI identified for each subject in the localizer stage, and from which activation during imagery was rewarded, was similar for all cue types. This was assessed by a one-way ANOVA test on the beta values extracted for each subject from the left motor ROI that was active during the Imagery-Reward stage ($F(2,51) = 0.01$, N.S.). Since the activation in this region was higher than baseline for all three cue types (Gain, Loss, and NS), the similarity in activation strength for the different cues may be due to a ceiling effect. Nevertheless, as we report below, its functional connectivity with the ventral caudate was higher for Gain than Loss, pointing to an alternative mechanism through which motivational cues might exert their invigorating effects.

Whole-Brain Analysis Comparing Gain versus Loss Activation

The whole-brain Gain versus Loss contrast during imagery trials was designed to detect additional brain regions that were sensitive to Gain versus Loss cues presented during the Cue-Imagery transfer test. This analysis yielded significant differential activity in right ventral caudate (11, 10, 6), right extended amygdala (26, 1, −6), right hippocampal complex (parahippocampus: 22, −23, −18; hippocampus: 20, 20, 06), as well as left inferior parietal lobule (IPL, −46, −65, 24) and right precuneus (11, −38, 42; see Figure 5A and Table S4). Note that the activation labeled here as extended amygdala may include nuclei of the basal forebrain as well (Alheid, 2003; see also Figure S4). In addition to the Gain and Loss cues, this stage also included a neutral cue (NS, a gray square in the middle of the screen), which was not presented in the previous Cue-Outcome stage. In all detected regions, Gain-related activations, but not Loss-related activations, were higher than NS (Figure 5B), indicating an invigorated imagery-related response during Gain compared to neutral cues. Paired t tests of Gain versus NS (not directly compared in the abovementioned contrast) yielded significant differences in ventral caudate, extended amygdala, and IPL.

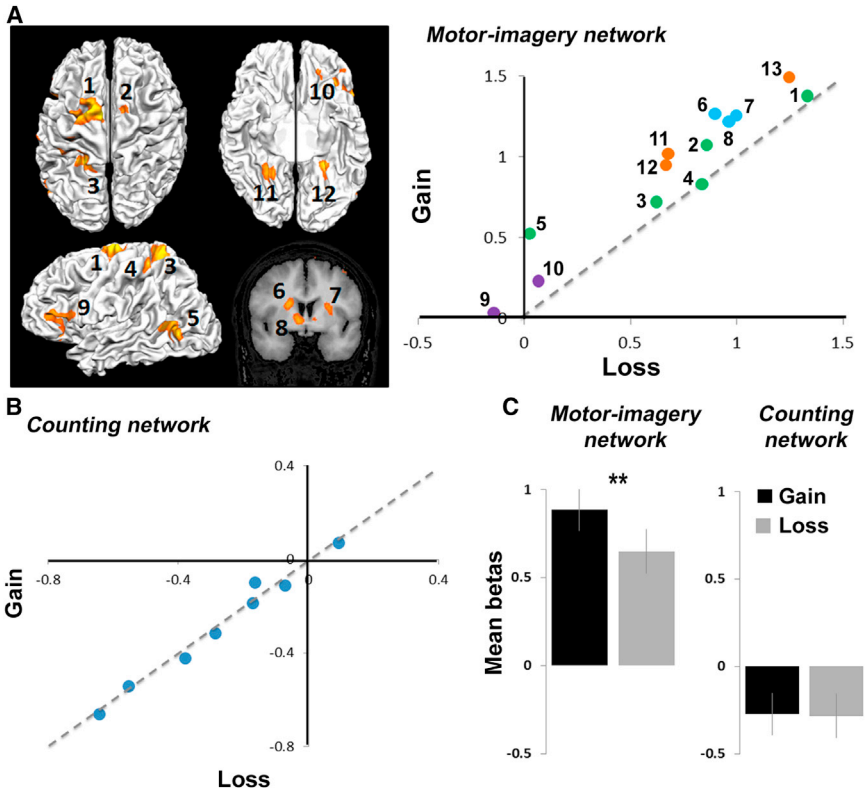

Figure 4. Gain- and Loss-Related Activation in the Motor-Imagery Network during the Transfer Test

(A) The motor-imagery network, as defined by prereward imagery versus counting contrast during the Imagery-Reward stage, is shown on the left on cortical surface maps and a coronal plane. Each dot in the scatter plot (right) represents the average beta values in an ROI within the motor-imagery network for Gain trials (y axis) and Loss trials (x axis). The number beside each dot corresponds to the brain area with the same number depicted on the brain images: 1, left precentral gyrus; 2, right precentral gyrus; 3 and 4, left superior parietal lobule; 5, left middle temporal gyrus; 6, right dorsal caudate; 7, left putamen; 8, right ventral caudate; 9, left inferior frontal gyrus; 10, right inferior frontal gyrus; 11 and 12, left and right fusiform gyrus, respectively; 13 (not shown on the brain image), right cerebellar tonsil (see Table S3 for details). Dots that fall above the dashed line indicate higher beta values for Gain than for Loss trials. Green dots refer to fronto-parietal and lateral temporal cortices, orange to inferior temporal and cerebellum, blue to basal ganglia, and purple to prefrontal cortex.

(B) The same analysis shown for brain regions that were more active during counting versus imagery trials in the Imagery-Reward stage (for statistical map see Figure S3).

(C) Bar graphs representing the mean beta values across all depicted brain regions for Gain (black) and Loss (gray) conditions, demonstrating overall higher activations for Gain versus Loss cues in the motor-imagery network ($t(13) = 6.57$, $p < 0.00005$) but not in the control "counting network" ($t(7) = 0.92$, $p = 0.38$).

Activations during Loss trials, however, were not significantly different than NS, implying that the Loss cue was not sufficient to induce a negative motivational state. Thus, regions in both reward and motor systems were more responsive to the presentation of the Gain CS during imagery, namely subcortical areas critical for signaling incentive salience (Cardinal et al., 2003), and regions in the parietal cortex involved in body representation and preparation of action (Cavanna and Trimble, 2006).

Functional Connectivity between Ventral Caudate/Extended Amygdala and Motor Cortex

Our finding of enhanced activation in extended amygdala and striatum resonates with previous human neuroimaging studies pointing to a role of these regions in cue-induced invigoration of instrumental behavior (Talmi et al., 2008; Prévost et al., 2012). It is possible that the effect that reward-related cues have on behavior, however, is mediated by enhanced synchronization of reward and instrumental (or in our case, motor imagery) circuits. To test this hypothesis, we examined whether the different Pavlovian cues affected the functional connectivity of the extended amygdala and ventral caudate (revealed in the whole-brain Gain versus Loss contrast) with motor regions that were activated by imagery. This analysis yielded enhanced functional synchronization during Gain versus Loss imagery trials between the ventral caudate and the ROIs detected in the Functional Localizer (left precentral gyrus) as well as right precentral gyrus (identified as part of the reward-imagery network), in the early trials of the test phase, gradually converging to similar functional connectivity values (Figure 6B). Importantly, this effect was not observed between the extended amygdala and these two motor regions (Figure 6C). ANCOVA tests substantiated these findings, yielding a significant interaction effect of cue × time when examining caudate-motor connectivity (ventral caudate-left precentral ROIs: $F(1,10) = 15.1$, $p < 0.005$; ventral caudate-right precentral gyrus: $F(1,10) = 25.9$, $p < 0.0005$), and no effect for the extended amygdala-motor connectivity (extended amygdala-left precentral ROIs: $F(1,10) = 0.65$, N.S.; extended amygdala-right precentral gyrus: $F(1,10) = 0.49$, N.S.). Subsequent analyses on the trial-by-trial activation estimates of each of these three regions over the course of the transfer stage revealed that in Gain trials, BOLD activation in both ventral caudate and motor cortex decreased over time, whereas the extended amygdala showed the opposite trend (see Figure S5). This implies that the diminishing functional connectivity effect was driven by reduced imagery-related and value-related activation over time. Taken together, synchronized activity among motor-imagery regions and the ventral caudate (but not the extended amygdala) was enhanced upon presentation of the Gain cue as compared to the Loss cue, primarily apparent in early phases of the transfer test.

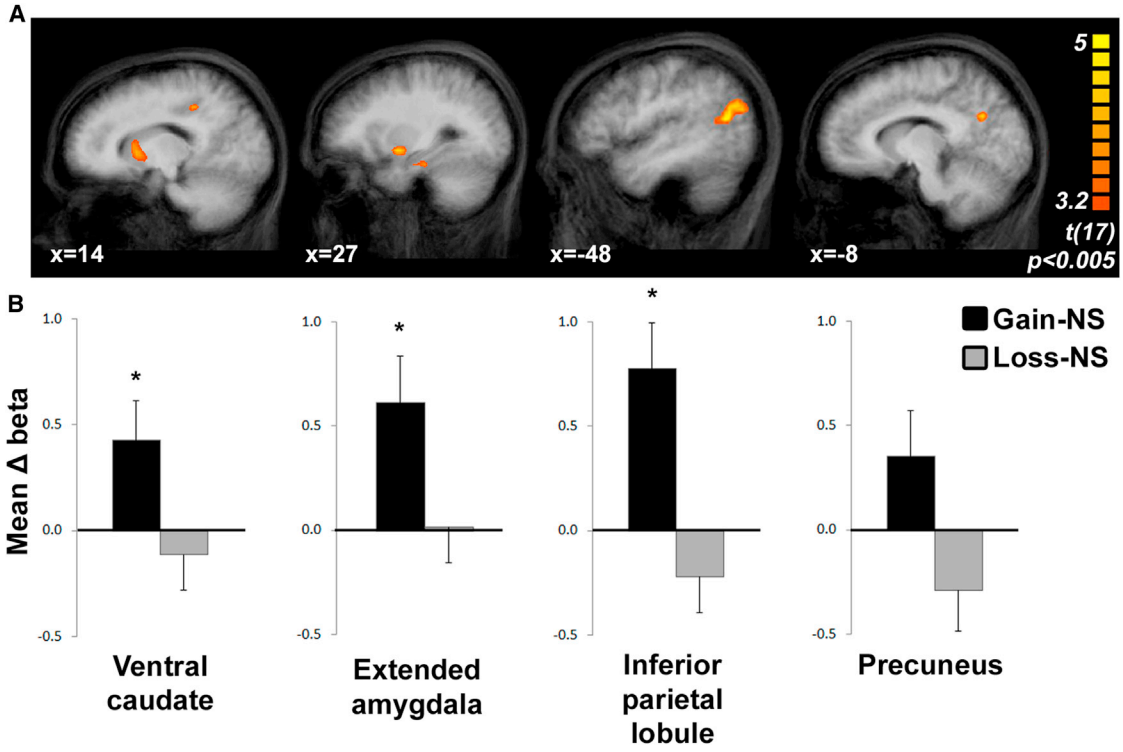

Figure 5. Whole-Brain Gain versus Loss Contrast during the Cue-Imagery Transfer Test

(A) Regions showing higher activation for Gain versus Loss trials during motor imagery in the Cue-Imagery transfer test. Statistical maps are overlaid on sagittal images of the groups' average anatomical scans. Higher Gain-related activations are observed in right ventral caudate (peak voxel x, y, z: 11, 10, 6), right extended amygdala (26, 1, −6; see also Figure S4), left inferior parietal lobule (−46, −65, 24), and precuneus (11, −38, 42).

(B) Bar graphs depicting differential beta values of Gain versus NS (black) and Loss versus NS (gray) in selected regions from the contrast map shown in (A). Paired t tests of Gain versus NS yielded significant differences in ventral caudate, extended amygdala, and inferior parietal lobule, whereas Loss versus NS comparisons were not statistically significant. Error bars represent SEM.

DISCUSSION

We show that reward-related cues learned in a Pavlovian manner enhance motor-imagery functions in the brain, resembling the way motivationally salient cues affect actual behavior. These findings may explain the sequence of neural processes by which environmental cues can significantly impact ongoing instrumental behavior. Participants initially performed a motor-imagery task in order to obtain monetary reward, a process that yielded activation in a distributed neural network termed here as the motor-imagery network. Next, participants acquired two Pavlovian associations, one between an initially neutral cue and monetary reward and another between a cue and monetary loss. In the critical Cue-Imagery transfer test, we examined neural responses induced by motor imagery in the presence of each of the cues learned in the Pavlovian stage. We found that the activation in the motor-imagery network as a whole, and primarily in the parietal cortex, was pronounced in the presence of the motivational cue (Gain) as compared to the loss-related cue (Loss). This effect was concurrent with increased activation in the ventral caudate and extended amygdala. We further observed enhanced functional connectivity between the ventral caudate (but not the extended amygdala) and motor regions activated by imagery during Gain but not Loss trials. This finding suggests the intriguing possibility that concurrent activation of reward and motor-imagery networks might mediate the influence that motivational stimuli exert on instrumental behavior.

The brain regions activated during the Imagery-Reward stage mirrored those that were detected in previous studies of motor imagery (Gallese and Sinigaglia, 2011; Willems et al., 2009), consisting of the precentral gyrus (primary motor cortex), superior parietal lobule, posterior temporal cortex, bilateral cerebellar regions, and striatum. The involvement of these regions in motor imagery is in line with the notion of overlapping functional neuroanatomy of motor imagery processes and actual motor action (Jeannerod, 2001; Lacourse et al., 2005; Munzert et al., 2009).

We demonstrated that a large portion of the motor-imagery network was affected by the presence of the reward-related cue during motor imagery, expressed by increased activation in the presence of this cue as compared to the loss-related cue. Thus, the appearance of the motivational cue presumably precipitated a motivational state (Corbit and Balleine, 2005; Homayoun and Moghaddam, 2009), affecting the activation in neural substrates underlying motor imagery and planning. In addition, we found that the presentation of the reward-related cue corresponded to increased functional synchronization between the ventral caudate and neural correlates of motor

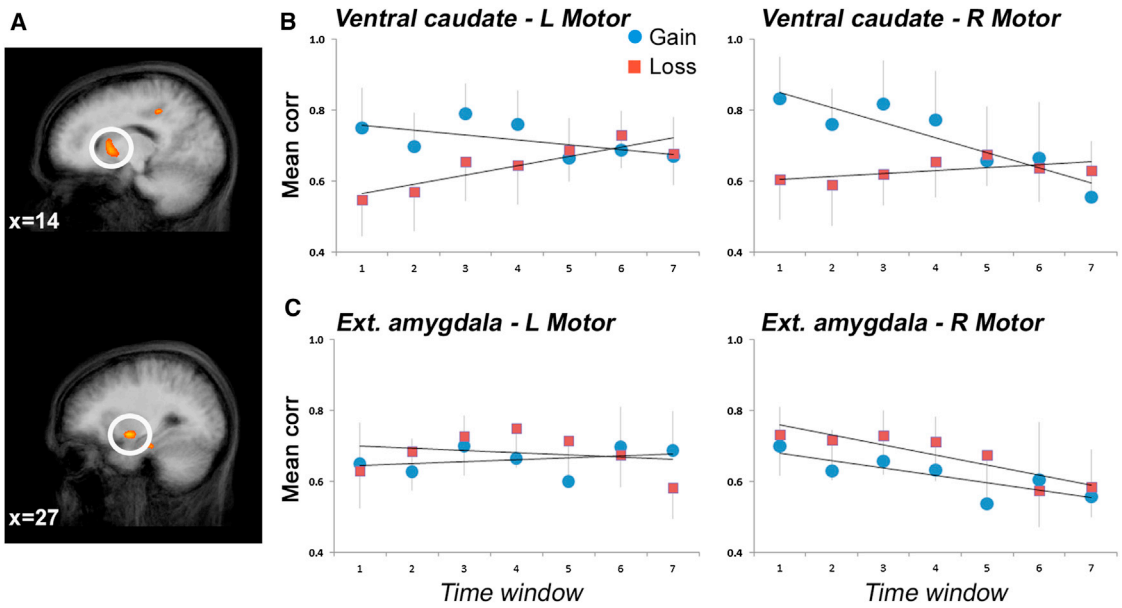

Figure 6. Functional Connectivity between Ventral Caudate/Extended Amygdala and Motor Cortex

(A) Depiction of the ventral caudate (top) and extended amygdala (bottom), which showed higher activation during Gain versus Loss imagery trials in the Cue-Imagery transfer test. Functional connectivity was computed by performing correlations between beta values from these areas and left and right motor cortex separately for Gain and Loss imagery trials.

(B) Mean beta-series correlations for Gain (blue circles) and Loss (red rectangles) between ventral caudate and left motor cortex ROIs (left) and right motor cortex (right) using sliding-window time bins (9 trials) depicting the dynamics of functional connectivity over time. Thus, each data point represents the mean correlation between beta values of two regions in a specific time window within the scan (i.e., trials 1–9, 2–10, 3–11 etc.; see details in Experimental Procedures). Both plots indicate that correlations between these regions were higher for Gain versus Loss trials, gradually converging to similar values (ANCOVA interaction effect using cue and time as factors—$F(1,10) = 15.1$, $p < 0.005$, and $F(1,10) = 25.9$, $p < 0.0005$, respectively).

(C) Same analysis as in (B) showing results of mean beta-series correlation values between the extended amygdala and motor cortices. No differences were detected in the correlation values between Gain and Loss conditions. Error bars represent SEM.

imagery. Previous neuroimaging studies have highlighted the involvement of motivational centers in mediating action contingency learning (Tricomi et al., 2004) and motivational effects on instrumental behavior (Bray et al., 2008; Talmi et al., 2008). Our findings may provide a broader account for the underlying mechanisms by which motivational cues can potentiate behavior. Specifically, we suggest that goal-directed behavior may be shaped via interactions among the ventral caudate, which signals the acquired motivational salience of presented cues, and motor regions, involved in the planning and execution of action. This mechanism, pending on further exploration, is important for establishing a fuller account of the chain of events that occurs between the presentation of motivationally salient cues and goal-directed behavior.

One could argue that the enhancement effect seen in regions of the motor-imagery network might be due to a general arousal effect that the motivational CS exerted on neural processing. We tackled this possibility by comparing the reward-related (Gain) CS to the Loss condition, which, due to its association with an aversive outcome, was expected to induce a similar, if not greater, state of arousal (indeed, during Pavlovian conditioning, the Loss cue induced greater SCR than the Gain cue). Second, we assessed the functional activation during motor imagery of brain networks not directly involved in motor imagery, namely those correlated with the counting task. These regions were apparently indifferent to the type of CS presented during motor imagery, thus excluding the possibility that the motivational CS simply enhanced activation throughout the brain. Notably, the mean activation level in the left precentral gyrus, from which BOLD signal was used as the criterion for reward delivery in the Imagery-Reward phase, did not appear to be affected by the presentation of the different CS types during the transfer test. In fact, the mean activation in this region was high in all three trial types (Gain, Loss, and NS), possibly representing a ceiling effect due to its extensive engagement in all imagery trials. Nonetheless, as discussed above, the functional connectivity between this region and the ventral caudate was affected by the presentation of CS type, suggesting an alternative invigoration mechanism by which motivational cues might exert their effects on the motor system.

Consistent with animal and human studies of PIT (Talmi et al., 2008; Prévost et al., 2012; for review, see Holmes et al., 2010), we demonstrate that the extended amygdala and striatum were activated to a higher degree during the presence of reward-related versus other cues. The ventral striatum and amygdala are critical to incentive learning, as they participate in assigning and subsequently signaling incentive value of stimuli and can thus influence goal-directed behavior (Cardinal et al., 2003; Everitt et al., 2003; Berridge et al., 2009; Corbit and Balleine, 2011). Animal studies have consistently shown that

lesions to ventral striatum and amygdala impair PIT (Hall et al., 2001; Corbit and Balleine, 2005), demonstrating the role of these regions in integrating motivational value with relevant instrumental responses (Cardinal et al., 2002; Holmes et al., 2010). An extension in our study to those conducted in animals, is the exploration of how motivational cues affect neural substrates of a covert process, which is not a measure of physical action. Our findings point to the involvement of similar brain regions in the transfer stage, supporting the notion that these regions are important for the motivational potentiation not only of actions but also of thoughts and action plans. Importantly, the activation patterns of these regions demonstrate an invigoration effect due to presentation of the reward-related cue above and beyond a suppression effect that the loss-related cue might have exerted. This is supported by significant increases in activation in these regions during Gain trials compared with the NS trials, and a (nonsignificant) decrease during Loss trials compared to NS.

In addition to reward-related regions, a whole-brain analysis comparing Gain versus Loss cues during imagery in the test stage yielded activations in the left inferior parietal lobule and precuneus. Both these areas play a central role in body representation as well as prediction and preparation of motor action (Blakemore and Sirigu, 2003; Lacourse et al., 2005; Cavanna and Trimble, 2006). That these regions were affected by the reward-related CS indicates that the incentive value of Pavlovian cues can alter covert motor processes in the absence of (though possibly affecting) actual performance.

Standard PIT paradigms were shown to be powerful models for explaining compulsive behaviors such as addiction, demonstrating that environmental cues associated with the object of addiction are profoundly important in precipitating drug seeking (Everitt et al., 2001). Compulsive behavior in humans, however, often involves cognitive features, such as conscious craving, intense imagery, and intrusive thoughts (Robinson and Berridge, 2008; May et al., 2010). Persistent intrusive thoughts and mental imagery related to rewarding outcomes can contribute to relapse in substance abuse and eating disorders characterized by poor impulse control (Pelchat, 2002; Pelchat et al., 2004; Robinson and Berridge, 2008). Our findings contribute to the understanding of how reward-related stimuli come to potentiate neural substrates of mental processes linking motivationally salient cues to action. As such, they may shed light on the sequence of events by which drug-paired cues exert influence over aspects of drug craving and relapse, particularly including conscious thoughts and imagery related to drug procurement. The ability to resist the involuntary signals associated with the rewarding value of addictive substances depends, among other things, on the recruitment of high-order cognitive resources to suppress such urges (Bechara and Van Der Linden, 2005). A fuller understanding of the neural mechanisms that support the translation of cue-related motivational salience to craving and subsequent relapse may be harnessed in order to attenuate these processes before they are transformed into actual drug-seeking behavior. We conclude that the use of rt-fMRI to gauge and reinforce brain activation associated with covert cognitive processes offers a tool for studying the undercurrents of such maladaptive behaviors.

EXPERIMENTAL PROCEDURES

Participants

Nineteen healthy, right-handed participants took part in this fMRI study (nine females, mean age 26.8 ± 3.4 years, range 23–36). One participant was excluded from analysis due to technical artifacts in image acquisition. The experimental protocol was approved by the Institutional Review Board of the Wolfson Medical Center, Holon, Israel. All participants had normal or corrected-to-normal vision, provided written informed consent, and were remunerated for their participation.

Task Description

In PIT, two associations are learned: a Pavlovian association between an initially neutral cue and a reward, and an instrumental association between a response and a similar reward. At the final stage, a test is performed to examine the influence of the cues on the behavior acquired in the instrumental stage (Everitt et al., 2001; Holmes et al., 2010). Here we used a similar protocol except that instead of physical behavior, participants performed motor imagery. Our experiment consisted of four stages, all performed during fMRI scanning: Functional Localizer, Imagery-Reward stage, Cue-Outcome stage, and Cue-Imagery transfer test (see Figure 1). In an attempt to increase motivation during imagery, participants were told prior to the experiment that they would earn between 100 and 160 shekels per participation, depending on their performance during the experiment.

Functional Localizer

This stage was designed to delineate an ROI for each subject, on which imagery conditioning would be performed (see below). During scanning, participants were instructed to engage in motor imagery when hearing the word "UP" and to count backward from 200 in steps of three when cued with the word "DOWN." They were asked to keep their eyes open at all times and were presented with a gray screen throughout. The Functional Localizer thus included two conditions—hand motor imagery (imagery) and backward counting (counting). Subjects were instructed before entering the scanner that in the imagery condition they should try to imagine themselves throwing a ball or a rock with their right hand without actually moving it (Johnson et al., 2012; Yoo et al., 2004). Participants did not receive any form of feedback regarding their success in recruiting motor-related brain activation (as in the Imagery-Reward stage described below). Throughout the scan, skin conductance response was gauged in order to verify that participants did not actually move their hands during imagery trials. Imagery and counting trials alternated six times each, imagery trials lasting either 20 or 24 s and counting trials 8, 10, or 14 s, randomly assigned. Scan duration was 3.5 min, which, based on pilot studies, was sufficient to extract a motor-imagery-related ROI. At the end of this stage, an ROI was delineated for each subject and was used in the following Imagery-Reward stage (for details on ROI extraction, see below).

Imagery-Reward Stage

This stage consisted of the same procedure and conditions as the Functional Localizer stage, except that participants were monetarily rewarded for increasing the BOLD signal in the ROI defined during the localizer stage. As in the Functional Localizer stage, the subjects' task was to engage in hand motor imagery when hearing the word "UP" (imagery) by imagining themselves throwing a ball or a rock with their right hand and counting backward when hearing the word "DOWN" (counting). The scan included alterations between imagery and counting trials (ten each), with imagery trials lasting either 20 or 24 s and counting trials 8, 10, or 14 s, randomly assigned. Before the onset of the experiment, participants read written instructions, explaining that successful motor imagery in the designated trials would yield a monetary reward of three Israeli shekels (approximately $1) and that they would hear a "ding" sound every time they received a reward. They were explicitly informed that any money they earned at this stage would be paid to them at the end of the experiment in addition to the payment for participating in the study.

Cue-Outcome Stage

In this stage, an association was formed between an initially neutral cue and monetary gain (Gain) and a different cue with monetary loss (Loss). Two initially

neutral stimuli were used in this stage (consisting of a striped or checkered pattern), randomly assigned to each subject as Gain and Loss cues. Prior to the onset of the scan, subjects read written instructions on screen, asking them to pay attention to the stimuli presented and to be aware of the relationship between the shapes and monetary outcome. Additionally, when presented with the word "Reward?" they were instructed to rate on a scale from 1 to 8 the degree to which they expected to receive a reward on the following screen (1—no expectation of reward, 8—certainly expecting reward). We used a partial reinforcement learning protocol with 40% contingency for each of the unconditioned stimulus (US) outcomes. Ratings and reaction times were recorded throughout.

Each trial lasted 6 s and consisted of three phases: cue presentation, subjective rating, and outcome (Figure 1B). At trial onset, one of the two CSs was presented in the center of the screen. After 1 s, subjects were prompted to rate whether the current CS was related to reward by pressing a button on one of two MRI-compatible response boxes containing four buttons each. Upon button press or after 4 s had elapsed, the outcome was presented on the screen until the end of the trial, indicating monetary gain ("you won 5 shekels"), monetary loss ("you lost 3 shekels"), or no gain/loss ("you did not win/lose"). The Gain outcomes were accompanied by an audio indication of a "ding" sound, identical to the one delivered for monetary reward in the Imagery-Reward stage. The Loss outcomes were accompanied by a different, lower sound. Each CS was presented 33 times (consisting of 13 CS-US (i.e., reinforced) trials and 20 CS-no US trials), with a jittered intertrial interval of 2–4 s, culminating in a 12 min scan.

Cue-Imagery Transfer Test

This stage was carried out devoid of reward (i.e., during extinction), so as to eliminate confounding effects of additional learning (Holmes et al., 2010). The task in this stage was identical to that of the Functional Localizer and Imagery-Reward stages, whereby participants were instructed to engage in motor imagery upon hearing the word "UP" (imagery) and count backward from 200 when hearing the word "DOWN" (counting). Trials alternated between motor-imagery segments (8 s) and backward counting (4–6 s). Critically, during each imagery trial, one of the Pavlovian cues presented in the previous stage was presented on screen (i.e., Gain or Loss), or a neutral stimulus (NS) not presented in the Cue-Outcome stage, consisting of a gray square. Prior to the scan, participants were presented with written instructions indicating that during the imagery trials, the cues from the previous stage would be presented on screen and were asked to keep their eyes open and look at the stimuli while performing the imagery task. This stage was thus comprised of four conditions—Imagery Gain (n = 15), Imagery Loss (n = 15), Imagery NS (n = 15), and Counting (n = 45), taking place in an ~10 min scan.

Skin Conductance Response during Motor Imagery

Throughout the experiment, skin conductance response (SCR) was recorded using BioPac skin conductance modules, monitored by AcqKnowledge software (BIOPAC systems). Before entering the scanner, MRI-compatible Ag-AgCl electrodes were placed on the participants' right hand on the thenar and hypothenar areas of the palm. SCR levels were determined taking the base-to-peak difference in waveforms (in micro siemens, μs) in the 0 to 8 s time window after stimulus onset. We used the SCR measurement as an indication of potential hand movements during the imagery and counting trials. To test for possible movements associated with motor imagery during Functional Localizer, Imagery-Reward stage, and Cue-Imagery transfer test, we performed a paired t test between SCRs during imagery versus counting. In all these stages, imagery SCRs were not different than counting SCRs (Functional Localizer: $p = 0.72$; Reward-Imagery stage: $p = 0.9$, Cue-Imagery transfer stage: $p = 0.87$).

Behavior Analysis of the Cue-Outcome Stage

Explicit Ratings of the Cue-Outcome Contingency

Explicit Pavlovian learning was assessed by separately calculating the average ratings of Gain and Loss trials. Since subjects' ratings were provided before trial outcome, all trials (i.e., trials that culminated in reward, losses, and null outcome) were analyzed. A linear regression analysis was performed on the groups' average rating scores, to examine the relationship between trial and average rating for each condition. Subsequently, an ANCOVA test was carried out to assess whether the learning slopes of Gain and Loss conditions significantly diverged over trials.

Skin Conductance Response during the Cue-Outcome Stage

SCR amplitudes to Gain and Loss cues were the dependent measures of appetitive and aversive conditioning, respectively. To avoid responses to reward or loss reinforcers, we only entered nonreinforced trials into analysis. The first two trials were excluded from statistical analysis to avoid arousal responses associated with startle. SCR levels were determined by taking the base-to-peak difference in waveforms (in micro siemens, μs) in the 0 to 8 s time window after stimulus onset. Subsequently, SCR levels for each condition were divided into early and late phases (trials 3 to 11 and 12 to 20, respectively) to examine conditioning-related SCR changes over time. The mean group scores for Gain and Loss were calculated and plotted. A two-way repeated-measures ANOVA test was performed on the data, using time (early/late) and CS (Gain/Loss) as factors, as well as separate t tests between Gain and Loss responses during early and late phases.

MRI Acquisition

Scanning was performed on a 3T Trio Magneton Siemens scanner located at the Human Brain Imaging Center of the Weizmann Institute of Science. During each fMRI scan, a time series of volumes was acquired using a T2*-weighted gradient-echo echo planar imaging (EPI) pulse sequence (TR 2000 ms, TE 30 ms, flip angle 80°, 37 oblique slices without gap, 20° from ACPC, 3 × 3 × 4 mm voxels). All images were acquired using a head coil (12 channels head matrix coil, Siemens Medical Solutions). In addition, T1-weighted high-resolution (1 × 1 × 1 mm) anatomical images were acquired for each subject with a magnetization-prepared rapid acquisition gradient-echo (MP-RAGE) pulse sequence (TE 2.98 ms, TR 2,300 ms, TI 900 ms, alpha 9°) to allow accurate 3D reconstruction and volume-based statistical analysis.

Real-Time fMRI

For carrying out the Functional Localizer and Imagery-Reward stages (described below), real-time fMRI analyses of acquired T2* images were carried out using Turbo BrainVoyager software (TBV, Brain Innovation). TBV analyzes incoming dicom files that are sent from the scanner computer to a designated computer every TR (i.e., every 2 s). The acquired data underwent motion correction and high-pass filtering for removal of low-frequency signal drifts during scanning.

Offline Analysis of fMRI Data

Offline preprocessing and data analyses of fMRI data were carried out using BrainVoyager QX version 2.10 (Brain Innovation), MATLAB, and software implemented in NeuroElf (http://neuroelf.net/). Images were corrected for slice timing, head movements, and linear drifts, and low frequencies were filtered out from the data. Images were spatially smoothed using a 6 mm full-width at half-maximum (FWHM) Gaussian kernel. The first two volumes (4 s) from the beginning of each scan were removed from the data set to allow for signal equilibrium. Functional and anatomical scans were spatially normalized by extrapolation into a 3D volume in Talairach space and resliced into isovoxel dimensions of 3 mm^3. Random-effects GLM analysis was performed on the group functional data. Conditions containing different trial types were convolved with the canonical hemodynamic response function and treated as predictors. In all the analyses, head motion parameters were included as regressors of no interest to account for motion effects. Cluster-size thresholding was performed using the "ClusterThresh" plug-in in BrainVoyager QX (Forman et al., 1995; Goebel et al., 2006).

Analysis of the Functional Localizer Stage

To enable the implementation of the Imagery-Reward stage, in which increases in imagery-related brain activity were associated with monetary reward, we initially set out to define an ROI involved in motor imagery. In order to monitor the differential activity associated with imagery versus counting, a contrast between these two conditions was carried out online during the localizer scan using an incremental GLM analysis updated every TR implemented in TBV software (Goebel, 2012). Upon scan termination, an ROI for each subject was delineated in the primary or secondary motor cortex using TBV, saving voxel coordinates that exceeded a threshold of $t > 3$, cluster size >5

consecutive voxels. These ROIs were used in the following Imagery-Reward stage by calculating their mean activation during motor imagery and rewarding the subjects accordingly (see below).

Offline Analysis

A random-effects group GLM was constructed, consisting of regressors of imagery and counting conditions. Subsequently, a contrast was performed between these two conditions, producing statistical maps at a threshold of $p < 0.0005$, cluster-size correction of $p < 0.05$, yielding a minimum cluster size of 23 voxels.

fMRI Analysis of the Imagery-Reward Stage

Online Analysis

The aim of the Imagery-Reward stage was to form an association between motor imagery and monetary reward (Bray et al., 2007). Thus, in imagery trials, the mean BOLD signal value of the ROIs defined in the preceding Functional Localizer stage was calculated by the TBV software and saved to file on a designated computer controlling the experiment. Every TR (i.e., every 2 s), within imagery trials, participants were monetarily rewarded in instances in which their ROI BOLD signal increased twice consecutively, conforming to the criterion—$[BOLD_{(TR)} > BOLD_{(TR-1)}]$ and $[BOLD_{(TR-1)} > BOLD_{(TR-2)}]$. Each time this criterion was met, participants heard a "ding" sound via their earphones, indicating that they had won three shekels. Taking into account the accumulative length of imagery trials, the hypothetical maximum winning each participant could receive was 160 Israeli shekels.

Offline Analysis

The purpose of this analysis was to identify the circuitry of regions involved in imagery conditioning. To assess activations associated with successful mental imagery, but not confounded with reward delivery, we constructed a GLM using a prereward imagery condition, a reward outcome condition, and a counting condition. The prereward imagery condition contained only the TRs that led to reward delivery (i.e., two consecutive TRs before participants received a reward). A second-level statistical analysis was performed, in which prereward imagery was contrasted with counting events, using a threshold of $p < 0.0005$, corrected for cluster size, $p < 0.05$, yielding a minimum cluster size of 21 voxels.

fMRI Analysis of the Cue-Imagery Transfer Test

The primary aim of the study was to examine whether and how motivationally salient cues affected motor imagery processes. First, a GLM was constructed, designed to identify regions that showed differential activity to Gain versus Loss cues during imagery, using the neutral stimulus (NS) as a reference condition. The GLM thus consisted of imagery trials that were performed during presentation of Gain, Loss, or NS, as well as a separate regressor for counting trials. The first two trials of each condition were discarded to avoid arousal responses associated with startle, and the condition regressors consisted of trial onsets (first TR). The remainders of the imagery trials (i.e., from TR 2 to trial termination) were modeled as a separate regressor.

Network-of-Interest Analysis

We first aimed at comparing Gain- and Loss-related activity in regions found in the motor-imagery network. To do so, we plotted the average beta values of each cluster in the network so that each data point represented the mean parameter estimates for Gain (y axis) and Loss (x axis) conditions. Data points accumulated above the diagonal line indicate higher average activity for Gain versus Loss. In addition, we compared the average activation across the network's regions between Gain and Loss events using a paired t test. To test whether results from this analysis were specific to the motor-imagery network, we performed the same analysis on a different set of regions, namely those that were more active during the counting task (as compared to imagery). As described above, for these regions too, mean beta values during imagery in Gain and Loss trials were displayed on a scatter plot and were also averaged and compared via paired t test.

Whole-Brain Analysis

In order to detect brain regions that were differentially activated to Gain versus Loss imagery trials, we performed a direct whole-brain contrast between Gain and Loss trials. From the resulting activated clusters, the average beta value of each condition (Gain, Loss, and NS) was extracted for each participant. For several of the regions, plots were formed depicting the difference between Gain versus NS and Loss versus NS.

Functional Connectivity Analysis

We next set out to examine whether the motor imagery ROIs (delineated in the Functional Localizer stage) showed differential functional connectivity patterns with ventral caudate and extended amygdala during the Cue-Imagery transfer test. In order to examine separately functional connectivity among regions for different event types, we computed separate parameter estimates for each individual trial in each condition and subsequently performed correlations among different regions for each condition separately (Rissman et al., 2004). Specifically, to detect dynamics of functional connectivity over the course of time, we computed correlations of beta values between pairs of ROIs using a sliding window so that correlations were performed on bins of beta values, starting from 1–9, 2–10, 3–11, etc. For each pair of regions (i.e., ventral caudate with motor cortices and extended amygdala with motor cortices), correlations were computed for each subject separately and then averaged across subjects. Correlations were computed separately for Gain and Loss trial. This enabled us to track changes in functional connectivity among ROIs for each condition separately throughout the scan. This analysis was performed between the ventral caudate and extended amygdala ROIs detected in the whole-brain contrast analysis and the single-subject ROIs from the Functional Localizer stage, as well as the right precentral gyrus delineated in the Imagery-Reward stage (Table S3).

Trial-by-Trial BOLD Activation Analysis

We next wished to examine the dynamics of activation over the course of the Cue-Imagery transfer stage in each of the three regions used in the functional connectivity analysis. We thus performed a linear regression analysis on each subject's trial-by-trial parameter estimates from the ventral caudate, extended amygdala, and motor cortex separately for Gain and Loss conditions. The slopes were averaged across subjects, and subjected to repeated-measures ANOVA, to detect potential differences among the regions in activation patterns across time in Gain and Loss condition.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.10.019.

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